Differences in the Urinary Metabolites of the Tobacco-Specific Lung Carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in Black and White Smokers[1]

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3. Abbreviations: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc, [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-ß-O-D-glucosiduronic acid; isoNNAL, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; HPB, 4-hydroxy-1-(3-pyridyl)-1-butanone; EtOAc, ethyl acetate; GC-TEA, combined gas chromatography-Thermal Energy Analyzer; GC-MS, combined gas chromatography-mass spectrometry; NNAL-TMS, trimethylsilyl ether of NNAL; HPLC, high performance liquid chromatography; CPD, cigarettes per day.

Abstract

Incidence and mortality rates for lung cancer in the U.S. are significantly greater in blacks compared to whites. This disparity cannot be explained by differences in smoking behavior. We hypothesize that the observed racial differences in risk may be due to differences in the metabolic activation or detoxification of the tobacco-specific lung carcinogen NNK. To test this, various biomarkers of NNK exposure and metabolism, including the urinary metabolites NNAL and, the presumed detoxification product, NNAL-Gluc, were examined along with questionnaire data on lifestyle habits and diet in a metabolic epidemiologic study of 61 healthy black and white smokers. Results demonstrated that the mean ratio of urinary NNAL-Gluc/NNAL, a likely indicator of NNAL glucuronidation and detoxification, was 30% greater in whites than in blacks. In addition, two phenotypes were apparent representing poor (ratio <6) and extensive (ratio ≥6) glucuronidation groups. The proportion of blacks falling into the former, potentially high risk group was significantly greater than in whites ($P<0.05$). The absolute levels of urinary NNAL, NNAL-Gluc and cotinine were also greater in blacks than in whites when adjusted for number of cigarettes smoked. None of the observed racial differences could be explained by dissimilarities in exposure or other sociodemographic or dietary factors. Also, it is unlikely that the dissimilarities are due to racial differences in preference for mentholated cigarettes as chronic administration of menthol to NNK-treated rats did not result in either increases in urinary total NNAL or decreases in NNAL-Gluc/NNAL ratios. Altogether, these results suggest that racial differences in the detoxification of NNK may, in part, explain the observed differences in cancer risk.

Introduction

Lung cancer rates in the U.S. vary greatly between races and, among the major racial/ethnic groups, blacks have the highest age-adjusted incidence and mortality rates (1,2). This racial disparity represents a significant public health problem with incidence rates varying from 122 per 100,000 for black males to 77.9 per 100,000 for white males (1). To date, there is little information available on the specific factors responsible for the observed differences in lung cancer between the races. Recently, it was suggested that socioeconomic differences alone could explain the differences in cancer rates, but no specific information on possible mechanism(s) was provided (3). While tobacco smoking is the major risk factor for lung cancer in both blacks and whites, it is unlikely that racial differences in tobacco smoke exposure can account for the differences in cancer rates. The proportion of ever smokers among blacks (57.7%) is the same as observed in whites (59.3%) (4). In addition, while the proportion of current smokers among blacks (40.4%) is slightly greater than among whites (32.9%), blacks tend to smoke far fewer numbers of cigarettes (4,5). Only 40.8% of black smokers are considered heavy smokers (≥15 CPD) compared to 70.9% for white smokers (4).

In the absence of differential exposures, it is possible that racial differences in cancer susceptibility are responsible for the differences in cancer incidence rates. However, few case-control studies on lung cancer risk comparing blacks and whites have been reported. An increased risk in blacks was observed for lung cancer in one small study (6). A similar difference in risk has also been observed for cancer of the esophagus, another tobacco-related site (7).

We hypothesize that the observed racial differences in cancer incidence are due to variations in carcinogen metabolism, resulting in differences in exposure of individuals and target tissues to activated tobacco smoke carcinogens. Of particular interest in the present study is the tobacco-

specific nitrosamine NNK[3]. Chemical and animal studies together with epidemiological observations provide strong evidence that NNK is a causative factor for lung cancer in smokers (8). NNK is found in substantial concentrations in tobacco smoke and induces adenocarcinoma of the lung in mice, rats and hamsters whether administered orally, topically or by s.c. or i.p. injection (8). Indeed, tumors are induced at total doses similar to those to which human smokers are exposed.

NNK requires metabolic activation to trigger its carcinogenic effects (9). Activation occurs through α-hydroxylation to reactive species which can methylate or pyridyloxobutylate DNA (Fig. 1). These DNA adducts have been detected in rodent and human tissues (10-14) and in mouse lung are associated with Ki-*ras* activation (15,16). These activated products of NNK metabolism can also form stable adducts with proteins such as hemoglobin (Hb) which have been proposed as possible biomarkers of internal exposure (17). In rats treated with NNK, 20-40% of the Hb adducts were released as 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) at levels which correlate with DNA-adducts and dose of carcinogen (18). Results in humans demonstrated increases in HPB-releasing adducts of >6-fold in snuff dippers and >2-fold in smokers over non-tobacco users (19).

The major metabolic pathway for NNK in most tissues is the conversion to NNAL by carbonyl reduction (Fig. 1) (9). Like NNK, NNAL is a powerful lung carcinogen in animal models and its carcinogenicity is dependent upon activation by α-hydroxylation. The majority of NNAL is glucuronidated to form NNAL-Gluc, a putative detoxification product of NNK and NNAL metabolism (20). In animal models, urinary total NNAL (NNAL + NNAL-Gluc) levels are highly correlated with dose of NNK (21).

Recently, a method was reported for the analysis of the two major NNK metabolites, NNAL and NNAL-Gluc, in urine of smokers (22) as well as non-smokers who have been passively exposed

to environmental tobacco smoke (23). In these studies, it was proposed that measurement of total NNAL could serve as a potential biomarker of NNK dose and metabolism. In addition, the molar ratio of NNAL-Gluc/NNAL may be indicative of an individual's capacity to glucuronidate NNAL. Since NNAL-Gluc is a probable detoxification product of NNAL and NNK, the ratio NNAL-Gluc/NNAL could serve as an index of detoxification capacity. Recently we reported on the intraindividual and interindividual variation in urinary NNAL and NNAL-Gluc levels in healthy smokers (24). Results verified the utility of NNAL-Gluc/NNAL ratio as a potential biomarker and suggested that phenotypic differences exist for NNAL glucuronidation capacity in human populations (24). Based upon these studies, 85% of smokers exhibited a low NNAL-Gluc/NNAL ratio (<6) and the remaining 15% exhibited a high ratio (>6). It was proposed that individuals in the low ratio group would have a relatively lower capacity to detoxify NNAL and NNK, and, thus, would be at greater risk for lung cancer. However, it must be noted that the confirmation of NNAL-Gluc as a detoxification product and the association of the low ratio phenotype with disease risk remain to be confirmed.

In humans, the balance between metabolic activation and detoxification of NNK will vary between individuals and is likely to affect one's risk for cancer upon exposure to NNK. Thus the goal of the present research was to utilize these newly developed biomarkers of NNK metabolism in black and white smokers to determine if racial differences in the metabolic activation or detoxification of NNK occur and can account, in part, for the observed differences in cancer incidence. Since differences in metabolism can result from either genetic or environmental factors, detailed questionnaire data on lifestyle habits, sociodemographics and diet were also obtained

Methods

Study subjects. All subjects were healthy adult smokers ranging in age from 21 to 50 yr. Subjects were recruited in the city of Mt. Vernon, New York, a community of 66,153 residents located 13 miles north of midtown New York City (25). In 1990, 55% of the population were black, 40% were white and 7% were of hispanic origin, and 9% of all families had incomes below the poverty line. Recruitment was carried out during 1992-1994 using fliers, public lectures, newspaper and television advertisements, recommendations from community leaders and word of mouth. To be eligible for participation, each subject was free of chronic disease, English speaking, and compos mentis.

Two interview clinics were estabilished in Mt. Vernon, one at Grace Baptist Church and the other at Mt. Vernon Hospital, at which all interviewing and sample collections were performed. After providing informed consent, subjects were administered a questionnaire providing detailed information on sociodemographics, tobacco and alcohol use, occupational, family and medical histories, environmental exposure, and lifestyle habits. In addition, a semi-quantitative NCI food frequency questionnaire (26) was administered to provide information on dietary intake.

Subjects were participating in a larger study of ethnic differences in carcinogen metabolism which included protocols for assessing caffeine metabolism. Thus, subjects were asked to fast and abstain from smoking starting at 12:00 A.M. and abstain from caffeine for 24 h. At 9:00 A.M. they were provided a cup of coffee and asked to collect their urine for the next 3 h. During this period, the questionnaire was administered and a blood sample was collected. Some subjects were recalled for a second analysis, 4-16 mo. after the first.

Collection of blood and urine. Urine samples were placed on ice immediately after collection and within 4 h, aliquoted and frozen at -20^{o}C until analysis. Blood was collected from an

antecubital vein into tubes containing EDTA as an anticoagulant and immediately placed on ice. Within 4 h blood was processed by centrifugation at 2100 x g for 15 min at 4°C and plasma was removed, aliquoted and frozen at -20°C. The remaining red cells were washed three times in ice cold 0.9% (w/v) saline and stored in the original blood tube at -20°C until analysis.

Analysis of creatinine and cotinine. Urinary cotinine was measured by GC-MS (27). In brief, cotinine was extracted from urine samples with CH_2Cl_2:isopropyl alcohol (80:20) containing 2% (w/v) ammonium hydroxide. Following evaporation of the organic solvent, cotinine was measured by GC-MS using deuterated cotinine as the internal standard.

Urinary creatinine was measured using a Kodak Ektachem 500 clinical chemistry analyzer.

Analysis of urine for NNAL and NNAL-Gluc. Urine was thawed and a 20-25 ml aliquot withdrawn for analysis as recently described by Camella et al. (24).

Analysis of HPB-releasing adducts. HPB-releasing adducts were analyzed by the method of Carmella et al. (19). HPB-releasing adducts levels were considered positive if levels were at least 2-fold greater than background levels. Previous results demonstrated that 20% of smokers are positive for HPB-releasing adducts (19). Thus, in the present study, HPB-releasing adducts were treated as a binary variable.

Effect of menthol on NNK biomarkers in F-344 rats. Male F-344 rats, –250 g each, were maintained on NIH-07 diet alone (control group, n=5) or on NIH-07 diet containing 5,000 ppm (-)menthol (menthol group, n= 5). All rats were given 2 ppm NNK in drinking water throughout the study. After 15 days, the rats were placed in metabolism cages and 24 h urine was collected. Blood (0.3 - 0.5 ml) was withdrawn from the orbital sinus. Urine was analyzed for NNAL and NNAL-gluc. Blood was analyzed for HPB-releasing hemoglobin adducts.

Statistical methods. Data are expressed as mean ± S.D. unless otherwise indicated. Comparison of group means were performed by Student's t-test or analysis of variance, followed by Scheffe's post-hoc test where appropriate. Correlations were determined by calculation of Pearson correlation coefficients. Prevalence data were compared by Chi-squared analysis.

Results

Study subject characteristics. A description of the study subject characteristics is provided in Table 1. Subjects ranged in age from 21 to 50 yr with a mean of 33.4 yr for blacks and 32.1 yr for whites. Overall, 48% of subjects were males and 52% females. Indicators of sociodemographic status were derived from questionnaire data (Table 1). There were no statistically significant differences in age, sex, ratio, marital status, education or number of residents per bedroom between the races.

A summary of smoking status parameters for the study subjects is provided in Table 2. No racial differences occurred in the mean age of starting smoking and total years smoking. Whites smoked on the average 8 more cigarettes per day (CPD) than blacks ($P<0.0003$) but smoked cigarettes of a slightly lower nicotine content ($P<0.0003$). Using CPD and tar and nicotine values per cigarette values, daily exposure of study subjects to tar and nicotine were estimated. Based on these calculations, whites were apparently exposed to 36% more nicotine and 48% more tar per day than blacks. The prevalence of smoking menthol cigarettes was significantly greater in blacks (73.5%) than in whites (18.5%).

Food frequency and alcohol intake data are provided in Table 3. No racial differences in intake were observed for any of the major food groups examined.

Urinary cotinine levels. Urinary cotinine levels were examined in all subjects (Table 4). The active smoking status of the study subjects was confirmed as cotinine levels ranged from 130 to 6770 ng/mg creatinine. Overall, cotinine levels were correlated with CPD ($r=0.32$, $P<0.006$). No racial differences were apparent in mean cotinine levels. However, when cotinine was expressed on a per CPD basis, mean cotinine was 2-fold higher in black than in white smokers ($P<0.0001$). Cotinine resutls were also calculated separately for smokers of 0-5, 6-10, 11-15, 16-20 and 21+ CPD (Figure 2). Cotinine levels were 75% greater in blacks compared to whites in the 16-20 CPD group, and 48% greater in the 21+ CPD group ($P<0.05$). No statistical differences were observed between the races for smokers of fewer than 16 CPD.

Urinary NNAL and NNAL-Gluc. Levels of urinary NNAL and NNAL-Gluc are summarized in Table 5. Concentrations are expressed on a per mg creatinine basis to account for urine dilution which differed between individuals. As observed previously (22,24) urinary levels of NNAL-Gluc are 2.5- to 4-fold greater than those of NNAL. While no racial differences were observed for NNAL-Gluc, NNAL levels were 2-fold greater in black subjects than in whites ($P<0.01$). A similar 2-fold increase in NNAL concentration was also observed when expressed on a pmol/ml urine basis. Mean total NNAL (NNAL + NNAL-Gluc) levels were 35% greater in blacks than in whites but the difference was not statistically significant ($P=0.13$).

In order to analyze urinary NNAL-Gluc and NNAL levels in relation to extent of exposure, levels of metabolites were expressed per CPD (Table 5). Significantly higher levels of all metabolites were observed in blacks compared to whites when expressed in this fashion ($P<0.05$). Total NNAL results were also calculated separately for smokers of 0-5, 6-10, 11-15, 16-20 and 21+ CPD (Figure 3). Total NNAL levels were 2-fold greater in blacks compared to whites in the 16-20 CPD group ($P<0.05$), and 2.7-fold greater in the 21+ CPD group. No differences were observed between the races for smokers of fewer than 16 CPD. A significant

correlation was observed between total NNAL and CPD for blacks ($r=0.38$, $P<0.02$) but not for whites ($r=0.22$).

In order to examine urinary NNK metabolites in relation to extent of internal exposure to tobacco smoke components, the relationship of NNAL-Gluc and NNAL with cotinine was also examined. In all subjects, cotinine levels were correlated with NNAL-Gluc ($r=0.39$), NNAL ($r=0.56$), and total NNAL ($r=0.48$). When NNAL-Gluc and NNAL were expressed on a per mg cotinine basis, racial differences were observed only for NNAL, where levels were 60% greater in blacks than in whites ($P<0.05$) (Table 5).

Racial differences in the metabolic ratio of NNAL-Gluc/NNAL. When NNAL-Gluc/NNAL ratios were calculated for the study subjects, significant racial differences were observed (Table 5). NNAL-Gluc/NNAL ratios were 42% greater in whites than in blacks. These ratios were unrelated to other smoking parameters including CPD and urinary cotinine levels.

Examination of the cumulative frequency distribution of ratios for all subjects revealed an apparent bimodal distribution with a break at a ratio of about 6 (Figure 4). Previous examination of this data by probit analysis clearly demonstrated two distinct groups consisting of individuals with high (>6) and low (<6) ratios (24). Thus, phenotypic differences were apparent in this population. When the data were analyzed in this fashion to examine racial differences, the percentage of subjects falling into the high ratio group was significantly greater among whites (26%) than among blacks (6%) ($P<0.05$) (Figure 5). To investigate the stability of these proposed phenotypes within individuals, a number of subjects were re-tested after 4-16 months. All subjects remained in the same phenotype category after re-testing suggesting that these phenotypes are consistent within indivduals over long periods of time (24). Likewise, no differences in phenotype were observed when 6 subjects were tested daily over a period of 3

days or when 24 h. urine samples were compared to random urine samples throughout the day (24).

HPB-releasing adducts. In an attempt to gain further information on the internal exposure of study subjects to the activated metabolites of NNK, HPB-releasing adducts were measured in blood samples from 48 participants (32 blacks and 16 whites). A total of 9 subjects (19%) were found to have HPB-releasing adduct levels at least 2-fold above background levels and were thus considered positive for the adduct. These values ranged from 2.3- to 5.0-fold greater than background levels. All positive samples were verified by repeat analysis. Adduct levels were low, ranging from 19 to 45 fmol/g Hb with a mean level of 27 fmol/g. There were no differences in the proportion of blacks testing positive compared to whites, however, a significantly higher proportion of males tested positive compared to females (Table 6). No relationship was observed between a positive HPB-releasing adduct status and urinary cotinine or total NNAL levels. However, individuals testing positive for adducts had significantly higher ratios of urinary NNAL-Gluc/NNAL ($P<0.01$). Differences observed in the proportion of subjects with the low NNAL-Gluc/NNAL phenotype in the 2 adduct groups were not statistically significant ($P=0.1$).

Menthol study in rats. Body weight data along with urinary NNK metabolite and blood HPB-releasing adduct levels in menthol-treated rats are provided in Table 7. Menthol supplementation did not appear to affect food intake as body weight was the same in the control and menthol groups. Increases of 24% for NNAL-Gluc levels and 32% for NNAL-Gluc/NNAL ratios were observed in menthol-treated animals. All other urine and blood measurements were unchanged.

Discussion

A key finding in this investigation is the racial difference in proportion of subjects falling into the high and low NNAL-Gluc/NNAL ratio phenotypes. In a previous report, we suggested that NNAL-Gluc/NNAL ratio may be an important factor in assessing the risk for cancer in smokers since NNAL-Gluc is a likely detoxification product of NNAL and NNK (21). Examination of the NNAL-Gluc/NNAL ratio data in this study suggests that two phenotypes exist representing a high ratio group (extensive glucuronidators) and a low ratio group (poor glucuronidators). Accordingly, individuals in the high ratio group may be partially protected against the carcinogenic effects of NNK and NNAL whereas those in the low ratio group may be at greater risk. The percentage of whites represented in this high ratio group was significantly higher than that of blacks. Thus, a greater proportion of blacks falling into the low ratio group may be indicative of a greater risk for lung cancer. Altogether, the results suggest that a decreased capacity to detoxify NNK and NNAL by glucuronidation may be one factor leading to increased risk for lung cancer in black populations.

The suggestion that NNAL-Gluc represents a detoxified product of NNK and NNAL results from glucuronidation being one of the major detoxification reactions for a wide variety of xenobiotics and carcinogens, resulting in products which are water-soluble and easily excreted. This mechanism is supported by results of a recent study in which F-344 rats were treated with NNAL-Gluc and excretion of urinary metabolites was examined (28). Of the total dose administered, almost 59% was excereted within 6 h and 94% after 24 h. Further, 76% of the dose was excreted as unchanged NNAL-Gluc and only 11% appeared as products of NNAL or NNK α-hydroxylation. In contrast, α-hydroxylation of NNK in F-344 rats is extensive, representing about 60% of dose, as determined by analysis of urinary metabolites (29,30).

The possibility must be noted that glucuronidation of NNAL could also serve as an active transport system delivering a small percentage of NNAL from the liver to the lung. Indeed, ß-glucuronidase activity has been reported in tissue samples of human lung (31). The notion that such ß-glucuronidase activity occurs *in vivo* was supported by the analysis of urinary products of NNAL-Gluc administered to rats in the above-mentioned study (28). While NNAL-Gluc itself represented the major urinary metabolite, 17% of the excreted products were NNAL, NNK or α-hydroxylation reaction products of NNAL or NNK. The occurence of these products while much lower than observed with NNK, nonetheless, suggests that a small percentage of NNAL-Gluc was converted to NNAL and, in turn, metabolized to activated products.

Given the different possible roles of glucuronidation reactions in carcinogenesis, DNA-adduct studies and carcinogenicity bioassays are required to confirm the role of NNAL-Gluc as a detoxification product. To date, such studies have not been reported.

The finding that blacks smoked, on the average, 8 fewer cigarettes per day than whites is consistent with other studies both in this communitiy (32) and elsewhere (4,5). Despite a lower exposure to tar and nicotine in black smokers, we observed higher urinary cotinine levels in blacks compared to white when adjusted for number of cigarettes smoked. These results were consistent with several previous reports (33,34) of serum cotinine in black and white smokers. In these studies, as well as the present report, there was no evidence for a reporting bias in either group. Altogether these studies suggest that racial differences in nicotine metabolism exist. Racial differences have previously been noted for the metabolism of a number of drugs and xenobiotics such as phenytoin (35), acetaminophen (36), and certain oral contraceptives (37). Differences in nicotine metabolism may account for the tendency of blacks to be light smokers: a similar internal exposure to nicotine is obtained from a fewer number of cigarettes smoked by blacks compared to whites. Such a phenomenon could be of particular importance in understanding the observed racial differences in success rates of smoking cessation programs.

An interesting important finding in this study is the observation that urinary NNAL levels are greater in blacks than in whites. These ethnic differences in NNAL as well as NNAL-Gluc and total NNAL are even greater when adjusted for number of cigarettes smoked. In fact, a correlation between CPD and total NNAL was observed in blacks only. Thus, like cotinine, NNAL exposure appears to be greater in black smokers than in white smokers. It is interesting to note that the differences for both cotinine and NNAL occur only for individuals smoking greater than 16 cigarettes per day. This relationship between cotinine and NNAL could be a result of a greater internal exposure to tobacco smoke per cigarette in blacks compared to whites, perhaps due to differences in smoking behavior. However, this is unlikely as NNAL/cotinine ratios were significantly greater in blacks than in whites. In addition, Wagenknecht et al (33) found no racial differences in serum thiocyanate levels in black *vs* white smokers, when quantity smoked was taken into account, despite finding significant differences in cotinine levels.

The significance of the finding of increased NNAL and NNAL-Gluc in the urine of black smokers relative to white smokers is unknown. One explanation is an increased overall exposure to NNK resulting in greater urinary excretion of metabolites. Alternatively, it can be argued that these changes are due to a more efficient conversion of NNK to NNAL and/or excretion of NNAL in urine, and may be indicative of a decrease in NNK activation. To clarify these questions regarding NNK and NNAL metabolism, it is important to account for all of the major NNK metabolites in urine. However, at the present time, methods have not been reported for the analysis of α-hydroxylation and N-oxidation products in the urine of human smokers.

In order to obtain information on the activation of NNK and NNAL, HPB-releasing adduct levels were measured in hemoglobin isolated from blood samples. As observed previously in smokers (19), only 20% of the subjects in this study had levels of HPB-releasing adducts significantly above baseline levels. No relationships between adduct status and race, total

NNAL levels or cotinine levels were observed. However, the presence of adducts was associated with higher NNAL-Gluc/NNAL ratios. It must be noted that for all subjects testing positive for adducts, the total adduct levels were low, several times lower than observed previously in smokers (19). The explanations for the low adduct levels and the relationship between adduct levels and NNAL-Gluc/NNAL ratios observed in this study are not known.

It is of interest to note that the use of mentholated cigarettes was greater in blacks (74%) than in whites (18%). Similar racial trends in mentholated cigarette usage have been observed previously (4,5). While these differences suggest that mentholated cigarette usage may be in part responsible for the increased risk for lung cancer in blacks, most case-control studies have found no increased risk of lung and oral cancers for users of mentholated cigarettes compared to non-mentholated cigarettes (38, 39). Another study found no effect of mentholation on the risk of lung cancer in men, whereas a slight increased risk was observed in women (40).

In order to examine the possibility that menthol exposure may alter NNK metabolism, urinary NNK metabolites and blood HPB-releasing adducts were measured in NNK-treated rats fed either control or menthol-supplemented diets. Menthol has little effect on NNK metabolites with the exception of a slight enhancement of NNAL-Gluc/NNAL raios and an increase in NNAL-Gluc excretion. Since the observed changes are inconsistent with the racial differences observed in the human study, it is unlikely that these differences can be attributed to use of mentholated cigarettes.

An important aspect in the present study is the subject recruitment strategy. Mt. Vernon, NY, represents an ideal community for such a study due to its equal representation of blacks and whites and relative racial equality in socioeconomic status. The Mt. Vernon community has been the site of previous biracial studies on lifestyle factors and health (32). This is particularly important in light of a recent study which suggested that differences in socioeconomic factors

can fully explain the observed racial differences in lung cancer incidence (3). The metabolic differences observed in the present study do not appear to be a result of differences in socioeconomic status based upon the questionnaire data obtained from the study participants. In addition, diet does not appear to be playing a major role as little differences were found in dietary factors examined. However, environmental influences cannot be discounted because of the relatively low power in this study to detect differences in dietary habits.

Concern has been expressed regarding the impact of heterogeneity among ethnic and racial subpopulations on studies dealing with the epidemiology of cancer in these groups (41,42). Indeed, it is recognized in the present study that metabolic differences are being examined among groups that are defined by political and cultural means in addition to ethnicity. However, despite the likely genetic heterogeneity, clear differences have been observed in cancer risk and, in this study, in metabolic parameters, that may be important in regulating risk. In light of the nature of the populations under study, cultural, dietary, behavioral and other like factors in addition to genetics must be addressed in identifying the underlying mechanisms responsible for the observed differences.

Altogether these results suggest that specific metabolic differences occur in the activation and/or detoxification of NNK between blacks and whites. Of particular interest is the finding of greater NNAL-Gluc/NNAL ratio in whites compared to blacks. While it is likely that high NNAL-Gluc/NNAL ratios are consistent with a decreased risk for lung cancer, case-control studies are required to confirm this hypothesis. Further studies of this nature are required before the differences noted in this study can be said to contribute to the racial differences in lung cancer risk.

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Table 1. Study Subject Characteristics

Variable	Blacks [a] (n = 34)	Whites [a] (n = 27)
Age (yrs)	33.4 ± 6.6	32.1 ± 9.1
Sex		
male	35.3	59.3
Female	64.7	40.7
Marital status		
single	50.0	59.3
married	29.4	37.0
divorced	8.8	3.7
separated	11.8	0
Education		
< 12 yrs	11.8	7.4
High school	17.7	14.8
College	55.9	48.2
Post graduate	14.7	29.6
No. residents/bedroom		
<1	13.3	20.0
1	46.7	40.0
2	26.7	32.0
≥3	13.3	8.0

[a] Values are percent except for age which are mean ± S.D.

Table 2. Smoking Status

Variable	Blacks [a] (n = 34)	Whites [a] (n = 27)
Age started (yr)	16.1 ± 4.6	16.4 ± 3.2
Total yrs smoking	17.0 ± 6.4	15.1 ± 8.3
Current CPD (#)	13.4 ± 5.7	20.9 ± 8.6 [b]
Nicotine/cig. (mg)	1.2 ± 0.2	1.0 ± 0.24 [b]
Nicotine/day (mg) [c]	15.9 ± 7.9	21.6 ± 12.7 [d]
Tar/cig. (mg)	13.9 ± 3.6	15.2 ± 2.7
Tar/day (mg) [c]	206 ± 104	305 ± 188 [e]
Menthol (%)	73.5	18.5 [e]

[a] Values are mean ± S.D.

[b] Significantly different from blacks, $P < 0.001$

[c] Calculated as follows: nicotine or tar/cig. x CPD

[d] Significantly different from blacks, $P < 0.05$

[e] Significantly different from blacks, $P < 0.01$

TABLE 3. Dietary Intake and Body Weight

Variable	Times/mo.	Blacks [a] (n = 34)	Whites [a] (n = 27)
Red meat	0	0	7.4
	1-10	14.7	22.2
	11-20	17.7	22.2
	>20	67.7	48.2
Fruit	1-10	5.9	18.5
	11-30	26.5	18.5
	31-40	5.9	7.5
	>40	61.8	55.6
Vegetables	1-10	5.9	11.1
	11-20	23.5	18.5
	21-30	26.5	18.5
	>30	44.1	51.9
Alcohol *(Whiskey equiv./wk)*		5.9 ± 6.9	6.3 ± 7.7
BMI (quartiles) [b]			
1		20.6	29.6
2		23.5	22.2
3		26.5	25.9
4		29.4	22.2

[a] Values are percent except for alcohol which are mean ± S.D.

[b] BMI = weight (kg)/height (cm)^2. No significant differences in BMI were found when calculated for each sex separately.

Table 4. Urinary Cotinine Levels

	Blacks [a] (n = 34)	Whites [a] (n = 27)
Cotinine		
(μg/mg creatinine)	1.65 ± 1.64	1.48 ± 1.07
Cotinine/CPD		
(μg/mg creatinine/CPD)	0.139 ± 0.099	0.071 ± 47.3 [b]

[a] Values are mean ± S.D.

[b] Significantly different from blacks, $P < 0.0001$

Table 5. Racial Differences in Urinary NNAL-Gluc and NNAL Levels

	Blacks [a] (n=34)	Whites [a] (n=27)
NNK Metabolites		
NNAL-Gluc (pmol/mg creatinine)	3.02 ± 3.32	2.53 ± 2.23
NNAL (pmol/mg creatinine)	1.22 ± 1.44	0.603 ± 0.345 [b]
NNAL-Gluc + NNAL (pmol/mg creatinine)	4.24 ± 4.56	3.13 ± 2.44
NNAL-Gluc/NNAL	3.11 ± 1.67	4.43 ± 2.60 [c]
NNK Metabolites Per Cigarette		
NNAL-Gluc/CPD (pmol/mg creat./CPD)	0.227 ± 0.231	0.136 ± 0.143 [b]
NNAL/CPD (pmol/mg creat./CPD)	0.092 ± 0.113	0.031 ± 0.024 [c]
NNAL-Gluc + NNAL/CPD (pmol/mg creat./CPD)	0.319 ± 0.332	0.167 ± 0.160 [c]
NNK Metabolites/Cotinine Ratios		
NNAL-Gluc/cotinine (nmol/mg)	2.11 ± 1.95	2.01 ± 1.52
NNAL/cotinine (nmol/mg)	0.754 ± 0.845	0.469 ± 0.209 [b]
(NNAL-Gluc + NNAL)/cotinine (nmol/mg)	2.87 ± 2.72	2.48 ± 1.64

[a] Values are mean ± S.D.

[b] Significantly different from blacks, $P < 0.05$

[c] Significantly different from blacks, $P < 0.01$

Table 6. HPB-Releasing Adduct Status

Variable	HPB-Releasing Adduct Status	
	Negative	Positive
N	39	9
Sex		
males	13 (33%)	7 (78%)
females	26 (67%)	2 (22%)
Race		
white	13 (33%)	3 (33%)
black	26 (67%)	6 (67%)
Cotinine [a] (μg/mg creatinine)	1.49 ± 1.51 (39)	1.34 ± 1.19 (9)
Total NNAL [a] (pmol/mg creatinine)	4.59 ± 4.82 (33)	2.19 ± 1.20 (7)
NNAL-Gluc/NNAL [a]	3.56 ± 2.36 (33)	6.07 ± 2.83 (7) [b]
NNAL-Gluc/NNAL < 6 (%)	87.9	57.1

[a] Values are mean ± S.D. (n)

[b] Significantly different from HPB-releasing adduct negative group, $P<0.01$.

Table 7. Effect of Menthol on NNK Metabolism in Rats

	Control [a]	Menthol [a]
Body wt. (g)	261 ± 6.9	259 ± 3.6
NNAL-Gluc (pmol/24 h)	1098 ± 121	1360 ± 129 [c]
NNAL (pmol/24 h)	1206 ± 121	1134 ± 1161
NNAL-Gluc + NNAL (pmol/24 h)	2304 ± 237	2493 ± 229
NNAL-Gluc/NNAL	0.91 ± 0.04	1.20 ± 0.08 [b]
HPB-released (pmol/g Hb)	76.4 ± 6.64	71.6 ± 7.21

[a] Values are mean ± S.D., n = 5.

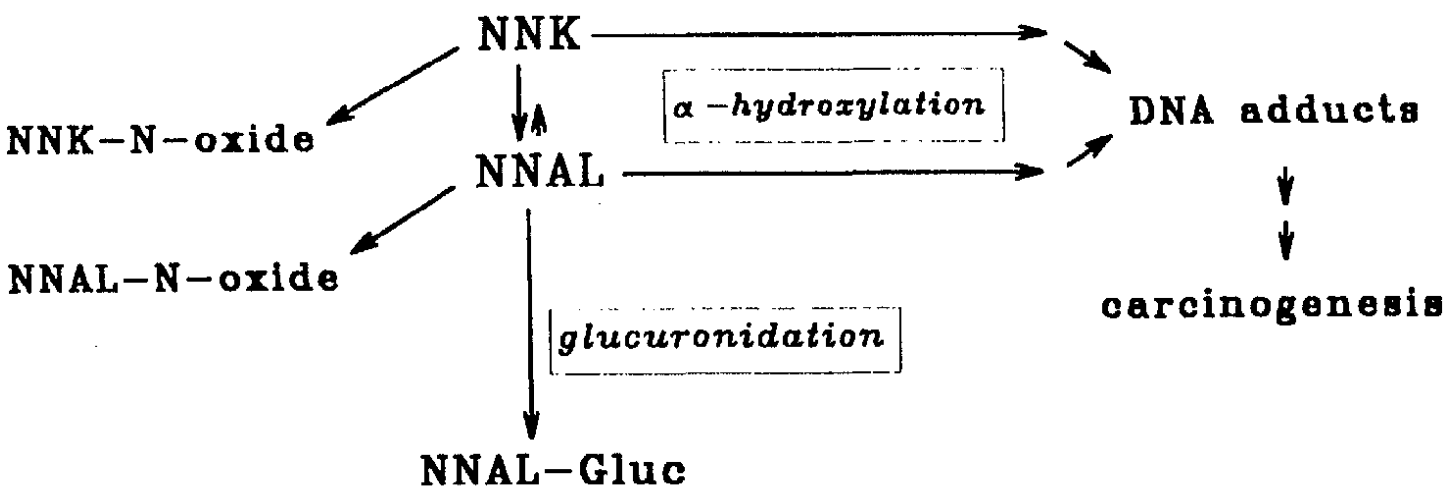
[b] Significantly different from controls, P = 0.0006.

[c] Significantly different from controls, P = 0.011.

Figure Legends

1. Major pathways of mammalian NNK metabolism.
2. Urinary cotinine levels by cigaretes per day in blacks and whites. Bars and error lines indicate mean ± SEM values and n values are provided in parentheses. Black/white differences in means are statistically significant for 16-20 and 21+ cigarettes per day groups ($P<0.05$).
3. Urinary total NNAL levels by cigarettes per day in blacks and whites. Bars and error lines indicate mean ± SEM values and n values are provided in parentheses. Black/white differences in means are statistically significant for 16-20 ($P<0.01$) and 21+ ($P<0.05$) cigarette per day groups.
4. Cumulative distribution of urinary NNAL-Gluc/NNAL ratios in all study subjects.
5. Racial differences in glucuronidation phenotype assessed by urinary NNAL-Gluc/NNAL ratios. Black/white differences in proportions are significant ($x^2 = 4.81$, $P<0.05$).

NNK METABOLISM



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